bio-protocol Preprint

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Sample collection, immunostaining and optical clearing

HM Hernán Morales-Navarrete YK Yannis Kalaidzidis FJ Frank Jülicher BMF Benjamin M Friedrich MZ Marino Zerial

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An abbreviated version of this protocol was published in eLIFE in Jun 2019

Liquid-crystal organization of liver tissue

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Detailed protocol

Fluorescence immunostaining - floating sections

A) Vibratome

Mold
1. Turn on the water bath at 60ºC.
2. Prepare 4% agarose in PBS and maintain it melted in the bath water at 60ºC. Put agarose in the plastic mold and add one piece of the dry liver at the bottom.
3. Make vibratome sections (100 µm thickness). Add PBS 500 ul/well in a 48 well plate. Just 1 slice per well.

B) Immunofluorescence

1. Remove the agarose from the tissue and permeabilize with 0.5% Triton X-100 in PBS (60 minutes) (300 ul/well).
2. Add primary antibody in TxBuffer (2 overnights room temperature)
3. Wash with 0.3% Triton/PBS (5 times 15 minutes)
4. Add secondary antibody + DAPI in TxBuffer (2 overnights room temperature)
5. Wash with 0.3% Triton/PBS (5 times 15 minutes)
6. Wash in PBS (3 times 1 minute)

C) M-SeeD Clearing.

1. Add 200 ul of 25% fructose for 4 hrs,
2. then 50% fructose for 4 hrs ,
3. 75% fructose ON
4. 100% fructose ON.
5. Add 200 ul of SeeD ON. All these steps are at room temperature.
6. Mount on a glass slide with SeeD solution. #1.5 coverslips (thickness 0.17 ± 0.005 mm). RI:1,49

- Different concentrations of fructose are prepared diluting 100% fructose with water.

Buffer recipes

TxBuffer (1L):
0.2% gelatin
300mM NaCl
0.3% Triton X-100
Add PBS to make 1L, aliquot to 50mL falcon tubes, store at -20˚C

Heat up PBS, gelantin and NaCl to dissolve. After that when the solution is under 40ºC add Triton.

Modified SeeDB clearing
Reagents

- M-SeeDB
80.2% (wt/wt) fructose, 0.5% 1-thioglycerol, ~0.1M phosphate buffer (pH7.5)*

- 100% Fructose pH7.5
100% (wt/v) fructose, 0.5% 1-thioglycerol, 0.1M phosphate buffer (pH7.5)

M-SeeDB recipe

Fructose	40.1 g
1M Na2HPO4	2.4 ml
1M NaH2PO4	0.6 ml
1-thioglycerol	0.15 ml
ddH2O	up to 50 g

100% Fructose

Fructose	40 g
1M Na2HPO4	3.2 ml
1M NaH2PO4	0.8 ml
1-thioglycerol	0.2 ml
ddH2O	up to 40 mL

* To keep fluorescent signal, the solution has to be buffered.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Morales-Navarrete, H. , Kalaidzidis, Y. , Jülicher, F. , Friedrich, B. M. and Zerial, M. (2020). Sample collection, immunostaining and optical clearing.

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2. Morales-Navarrete, H., Nonaka, H., Scholich, A., Segovia-Miranda, F., de Back, W., Meyer, K., Bogorad, R. L., Koteliansky, V., Brusch, L., Kalaidzidis, Y., Jülicher, F., Friedrich, B. M. and Zerial, M.(2019). Liquid-crystal organization of liver tissue. eLIFE. DOI: 10.7554/eLife.44860